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Cochlear NMDA receptor blockade prevents salicylate-induced tinnitus

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Abstract. *Cochlear NMDA receptor blockade prevents salicylate-induced tinnitus.* Large doses of aspirin produce reversible hearing loss and tinnitus. These effects have been attributed to the salicylate ion, the active component of aspirin. Salicylate acts as a competitive antagonist at the anion-binding site of prestin, the motor protein of sensory outer hair cells. This provides an explanation for the hearing loss induced by aspirin. However, the molecular mechanism of salicylate-induced tinnitus remains obscure. One physiological explanation is that salicylate ototoxicity is likely to originate in an alteration to arachidonic acid metabolism. Arachidonic acid potentiates NMDA receptor currents. We therefore tested the involvement of cochlear NMDA receptors in the occurrence of tinnitus. Tinnitus was assessed with a behavioural test based on an active avoidance paradigm. Results showed that the tinnitus induced by salicylate may be suppressed by the introduction of NMDA antagonists into the cochlear fluids. To determine if the activation of NMDA receptors was linked to cyclooxygenase inhibition, we investigated the effect of mefenamate (a potent cyclooxygenase inhibitor). Since NMDA antagonists also blocked mefenamate-induced tinnitus, we suggest that salicylate-induced tinnitus is mediated by cochlear NMDA receptors through the inhibition of cyclooxygenase activity. Target cochlear NMDA receptors may therefore present a therapeutic strategy for the treatment of tinnitus.

Introduction

In industrialised nations, 8 to 10% of the adult population currently experience tinnitus (ringing in the ears). This amounts to approximately 20 million people in the United States of America. Tinnitus is the subjective perception of a sound in the absence of a corresponding external source. This corresponds to an abnormal sound which is clearly perceived in quiet surroundings and which very often becomes highly debilitating. Constantly perceiving a sound, without having any way to make it cease, implies a state which can be highly anxiogenic, and which may severely impair the quality of life.[1-4] Although there are some palliative treatments (maskers, behavioural therapy...), no real and specific treatment is available. This is due to the sparsity of evidence for a physiological basis of tinnitus from clinical observations and from animal experiments.

Salicylate, which is a member of the nonsteroidal anti-inflammatory drugs (NSAIDS) family, is widely recognised for its antipyretic, analgesic and anti-inflammatory properties. It has been clear for more than a century that a large dose of salicylate produces hearing loss and tinnitus in humans.[5] Both hearing loss and tinnitus develop over the initial days of treatment, and are reversible within a few days of cessation of treatment. These effects of salicylate have also been demonstrated in animals.[6-8] In humans, the pitch of salicylate-induced tinnitus appears to be in the high-frequency range in almost all cases.[9] In rats, salicylate induces tinnitus with a frequency close to 10 kHz.[6-8] Salicylate-induced hearing loss is caused by the blockage of prestin, the molecular motor of outer hair cell electromotility.[10-11] By contrast, the site and the mechanism of the generation of the tinnitus induced by salicylate remains unclear.

Electrophysiological studies report that the injection of salicylate increases spontaneous activity in single units of the auditory nerve[12,13] and modifies the average spectrum activity recorded from the round window, which is a gross measure of spontaneous activity of the auditory nerve.[14] The characteristics of these changes appear to be similar to the characteristics of salicylate-induced tinnitus in animals.[14] This suggests that, at least in part, tinnitus induced by salicylate is associated with a dysfunction of neurotransmission within the cochlea.

Measuring tinnitus in animals

Validating a behavioural procedure to assess the presence of

tinnitus in animals is an unusual and difficult task. Jastreboff and colleagues developed the first behavioural model for tinnitus in rats. The protocol was a conditioned suppression paradigm based on water deprivation.[6,7] Animals were trained to stop drinking whenever the broadband noise was turned off by pairing its absence with foot shocks. One major feature of Jastreboff's protocol is that animals have to be kept thirsty, leading to a loss of body weight up to 20%. When the loss of body weight was reduced, salicylate-induced modifications of behaviour were drastically reduced. This led to the suggestion that changes in physiological state strongly affect the motivational level of the animals, making the test difficult to interpret in terms of tinnitus.[6]

To avoid changes in the physiological state of the animal, we recently designed a new behavioural model based on an active avoidance paradigm.[8] Tinnitus was assessed with an active avoidance paradigm. Animals had to perform a motor task (i.e. jump on a climbing pole) when hearing a sound matching salicylate-induced tinnitus (10 kHz). Salicylate treatment provoked a progressive decrease in the score and a concomitant development of hearing loss, as demonstrated by CAP threshold recordings.[8] When the intensity of sound eliciting behavioural responses was adjusted as a function of CAP threshold shift, no significant decrease in the score was observed. In conjunction with the striking similarity between the time pattern of CAP threshold shifts, DPOAE recordings and score measurements, these results demonstrate that the score was linked to hearing performance. By contrast, salicylate treatment drastically pushed up the number of false positive responses. Because they have a sound hallucination (i.e. tinnitus), salicylate-treated animals are more likely to execute the motor task during the silent periods. Animals treated with salicylate will therefore behave as if they hear a sound when no external sound is present. Accordingly, results demonstrated that treating animals with salicylate significantly increased the number of false positive responses (jumping on the climbing pole) during silent periods.[8] In other words, the number of false positive responses is a powerful indicator of the occurrence of tinnitus.

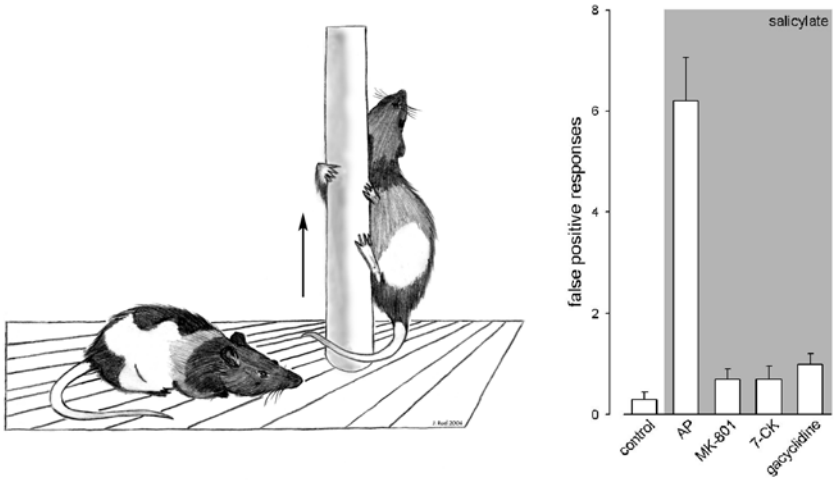

Figure 1
Molecular mechanisms of salicylate-induced tinnitus
Animals were conditioned to jump on a climbing pole in response to a sound stimulus. The false positive responses (i.e. jumping during silent periods) is a good indicator of the occurrence of tinnitus. Intraperitoneal saline injections did not provoke the occurrence of false positives. By contrast, intraperitoneal injections of salicylate (300 mg/kg/day during 4 days, grey area) significantly increased the number of false positives. The histogram shows the number of false positive responses measured at day 4 in animals injected with saline solution (saline), and in animals injected with salicylate + gel foams soaked in artificial perilymph only (AP, n = 10) or NMDA antagonist MK-801 (10 µM, n = 10), 7-chlorokynurenate (7-CK, 50 µM, n = 10), or gacyclidine (50 µM, n = 10). When compared with AP alone, the local application of MK-801, 7-CK, or gacyclidine significantly ($p < 0.001$) reduced the occurrence of the false positive responses (Figure adapted from Guitton[8]).

Molecular mechanisms of salicylate-induced tinnitus

Salicylate has been shown to inhibit cyclooxygenase activity, leading to an increase in intracellular arachidonic acid. To determine whether salicylate-induced tinnitus is linked to cyclooxygenase inhibition, we investigated the effect of mefenamate (a potent cyclooxygenase inhibitor), and found that mefenamate treatment does indeed result in a significant increase in the number of false positive responses (Figure 1), indicating that inhibition of the cyclooxygenase pathway is one of the mechanisms by which salicylate and mefenamate induce tinnitus.[8]

The local administration of three different NMDA antagonists (7-chlorokynurenate, gacyclidine, and MK-801) in perilymphatic fluids through a gel foam placed on the round window of each cochlea blocked the occurrence of both salicylate- and mefenamate-induced tinnitus (Figure 1). Salicylate-induced tinnitus is therefore mediated by cochlear NMDA receptors through the inhibition of cyclooxygenase activity.[8] The results discussed here provide evidence for a new pharmacological effect of salicylate in inner ear physiology. In addition to reducing OHC electromotility, salicylate may act on cochlear fast synaptic transmission through the activation of NMDA receptors, accounting for the occurrence of tinnitus.

Salicylate mechanisms in other types of tinnitus

In the cochlea, normal synaptic transmission between inner ear cells and primary auditory neurons is mediated by the AMPA receptor.[15] However, analysis with gene expression, immunocytochemistry and *in situ* hybridisation indicates that the cochlea expresses NR1 and NR2A-D subunits of NMDA receptors.[16,17] Although these NMDA receptors are not involved in cochlear synaptic transmission, they are involved in synaptic repair after excitotoxicity[18] and NMDA antagonists protect sensory hair cells from aminoglycoside ototoxicity,[19] as well as preventing excitotoxicity induced by cochlear ischaemia and acoustic trauma.[20-22] Although experiments are needed to confirm the involvement of cochlear NMDA receptors in other models of tinnitus (noise trauma, ischaemia, aminoglycosides or cisplatin ototoxicity), NMDA antagonists may constitute an attractive candidate for the treatment of tinnitus in humans.

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